Asian-Aust. J. Anim. Sci.
Vol. 21, No. 1 : 19 - 24
January 2008

www.ajas.info

The Expression of Genes Related to Egg Production in the Liver of Taiwan Country Chickens

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ABSTRACT : The purpose of this study was to detect expression of genes related to egg production in Taiwan Country chickens by suppression subtractive hybridization. Liver samples of mRNA extraction from two Taiwan Country chicken strains (L2 and B), originated from the same population but with very distinct egg production rates after long-term selection for egg and meat production respectively. Two-way subtraction was performed. The hepatic cDNA from the low egg production chickens (B) was subtracted from the hepatic cDNA from the high egg production strain (L2). The reversed subtraction (L2 from B) was also performed. The resulting differentially expressed gene fragments were cloned and sequenced. We sequenced 288 clones from the forward subtraction and 96 clones from the reverse subtraction. These genes were subjected to further screening to confirm the differential expression between the two genetic breeds of chickens. The apolipoprotein B (apoB) was expressed to a greater extent in the liver of the L2 than in the B line chickens. The 5-aminoimidazole- 4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (PURH) was expressed to a greater extent in the liver of the B than in the L2 strain chickens. We demonstrated that both apoB and PURH were more highly expressed in the liver than that in other tissues (muscle, ovary, and oviduct) in laying Taiwan Country chickens. Taken together, these data suggest that after the selection for egg production, expression of apoB and PURH genes were also changed. Whether the changed expression of these genes is directly related to egg production is not known, but these two genes may be useful markers for egg laying performance in Taiwan Country chickens. (**Key Words :** apoB, Egg production, Liver, PURH, Taiwan Country Chicken)

INTRODUCTION

The Taiwan Country chicken is one of the major meat type chickens in Taiwan. It provides about half of the chicken meat for the market. Compared to broiler meat, the flavor and texture of Taiwan Country chicken meat are favored in Taiwan. One of the problems with production of the Taiwan Country chicken is that its egg production rate is much less than that of the Leghorn layers or broiler breeders (Lee, 2006). Lee et al. (1997) reported two strains (B and L2) of Taiwan Country chickens that were selected from the same population for more than 20 generations resulting in very different egg production rates. The L2 line chickens have a much better egg production rate, but grow slower than the B line chickens (Yen, 2004). The hepatic gene expression in Taiwan Country chickens is affected by caponization (Chen et al., 2006).

There are 78 chromosomes with a haploid content of 1.1×10^9 base pairs (bp) of DNA in chickens. The first draft of the chicken genomic DNA sequence was presented in 2004 by the National Human Genome Research Institute (NHGRI). Due to the complexity of the chicken genome, a high percentage of the sequences is still missing. There are studies of expressed sequence tags (EST) in chickens (Spike et al., 1996; Abdrakhmanov et al., 2000; Tirunagaru et al., 2000; Carre et al., 2006). These chicken EST clones have contributed greatly to the understanding of the chicken genome.

Although many genes from the chicken liver have been studied, an egg-laying performance related liver cDNA population has not been reported. Although differential gene expression between different breeds of chickens was reported (Sun et al., 2005), expression of genes related to laying ability were not studied. We conducted this

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Received January 6, 2007; Accepted April 26, 2007

experiment to clone genes related to high egg production traits in Taiwan Country chickens and utilized differential screening and Northern blotting techniques to discover genes related to egg-laying performance.

MATERIAL AND METHODS

Animals

The Taiwan Country chickens were raised in individual cages on a commercial layer diet. To detect expression of genes related to egg production performance in Taiwan Country chickens, liver samples from two Taiwan Country chicken breeds (L2 and B lines) with very distinct egg production rates were taken. Eight chickens (4 birds from each line) were used for the gene expression study. The egg production rates at the age of 34-wk for L2 and B chickens were 90.8±5.2% and 44.7±9.0%, respectively. The average body weights at the age of 34-wk for L2 and B chickens were 2.04±0.33 kg and 2.30±0.33 kg, respectively. The oviposition time for each bird was observed for a week in order to estimate the oviposition time of each bird on the sampling day. On the day of sampling, the chickens were fed 2 h before the birds were killed. Liver samples were taken in the postprandial condition with the eggs in the shell gland. The protocol was approved by the Experimental Animal Management and Use Committee at National Chung Hsing University. Liver and other tissues were quickly dissected, frozen in liquid nitrogen and stored at -70°C until RNA extraction.

Extraction of RNA

Total RNA was extracted by the guanidinium-phenol-chloroform extraction method (Chomczynski and Sacchi, 1987) with minor modifications (Hsu et al., 2004; Wang et al., 2004). The quality of the RNA was monitored by examination of the 18S and 28S ribosomal RNA bands after electrophoresis. The RNA was quantified by spectrophotometry at 260 nm and stored at -70°C.

Suppression subtraction hybridization (SSH)

The SSH procedure utilized the PCR Select kit from Clontech (BD Biosciences, Mountain View, CA 94043). Procedures were described in detail by Chang et al. (2007) and Yen et al. (2006). Briefly, mRNA from the livers of the two lines of chickens (L2 and B lines) were each reversely transcribed and double stranded cDNA was synthesized. After RsaI restriction enzyme digestion, the Tester DNA (cDNA from L2 line) was divided into two portions and each was ligated with Adapter 1 (Tester 1) or Adaptor 2 (Tester 2). The Driver DNA (cDNA from the B line) was not ligated with any Adaptor. Tester 1 or Tester 2 cDNA were denatured at 98°C for 1.5 min and hybridized with denatured Driver cDNA at 68°C, as described by Wang et al. (2006). After hybridization, any single strand DNA with Adaptor 1 or Adaptor 2 represented genes expressed in the liver of L2 but not in B layers. The single stranded DNA without Adaptors represented genes expressed in the liver of B but not in L2 layers. The Adaptor 1 and Adaptor 2 populations were pooled for a second hybridization with fresh denatured Driver Cdna. Any resulting double stranded DNA molecules with both Adaptor 1 and 2 represented gene sequences preferentially expressed in the L2 line. Those molecules were amplified after a 14-cycle PCR using a pair of nested primers containing sequences from Adapter 1 and 2. The differentially expressed gene fragments were then cloned into pGEM-T Easy TA cloning vector (Promega, Madison, WI53711). The resulting clones were selected for sequence analysis by a genetic analyzer (ABI 3730, Applied Biosystems, Foster City, CA 94404). The reverse subtraction (L2 from B) was also performed. We selected 288 clones from forward subtraction and 96 clones for the reverse subtraction for differential screening analysis. The genes selected by differential screening were subjected to Northern analysis to confirm the differential expression of genes between the two genetic breeds of chickens.

Differential screening

The differential screening procedure followed the procedure described by Clontech PCR-Select Differential Screening Kit user manual (BD Biosciences, Mountain View, CA 94043). The detailed procedure was described by Yen et al. (2006). In brief, the transformed *E. coli* were transferred to a nylon membrane and grown on a LB agar plate. The membrane containing the bacterial colonies was incubated for 12 h at 37°C. The membrane was then treated with 10% sodium dodecyl sulfate solution for 5 minutes before being treated with the denaturing solution (1.5 M NaCl, 0.5 M NaOH) for 5 minutes. The membrane was then neutralized with neutralizing buffer (1.5 M NaCl, 0.5 M Tris-HCl at pH 7.4) for 5 minutes. The DNA on the membrane was UV-crosslinked and baked at 80°C for 30 minutes. The membranes were then used for hybridization to detect differentially expressed genes. The DNAs remaining after forward subtraction and reverse subtraction were used as probes for the screening.

Northern analysis

Total RNA (20 μg of each sample) was electrophoresed and transferred to nylon membranes (Cheng et al., 2006). The membrane was prehybridized at 42°C in UltraHyb (Ambion, Austin, TX) for 1 h and then the denatured cDNA probe (95°C for 5 min) was added at a concentration of 1 pM, to hybridize with the targeted gene transcripts overnight at 42°C. The probes were generated by PCR amplification of gene fragments reported in this study with adapter sequences as primers. Hybridization results were quantified by phosphor-image analysis as previously described (Ding et al., 1999; Yen et al., 2005). The

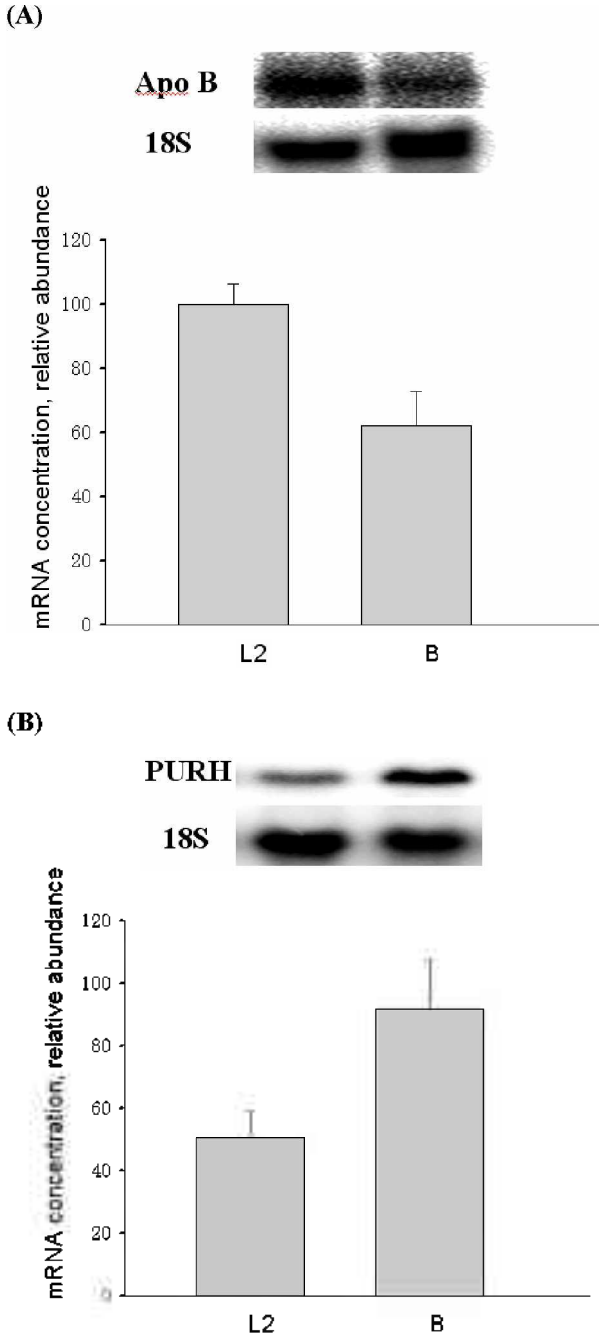

Figure 1. The differentially expressed hepatic genes in B and L2 Taiwan Country chickens. These genes were discovered by a suppression subtractive hybridization technique. The mRNA concentrations of apolipoprotein B and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (PURH) were determined by Northern analysis. Probes were generated by random priming procedures. The values were the average of five birds. (A) mRNA concentration of apolipoprotein B (apoB); (B) mRNA concentration of PURH.

densitometric value for an individual transcript in a sample lane was normalized to the densitometric value for the 18S ribosomal RNA in the same lane. The relative mRNA abundance of chicken genes was estimated by the densitometric value.

Statistical analysis

The data were analyzed using a Student's t test to evaluate the treatment effect (selection for egg laying performance) using SAS statistical software (SAS Institute, 2001). The significant level between treatments was set at 5%. The mean and SE for each transcript are presented.

RESULTS

Differentially expressed genes from SSH experiments

Two way subtractions, both forward and reverse were performed and many differentially expressed gene fragments were revealed. For the forward subtraction, 288 gene fragments, preferentially expressed in the L2 line, were sequenced. For the reverse subtraction representing the B line, we sequenced 96 gene fragments. Because there were many false positive clones for either direction of subtraction, the gene fragments were blotted on to membranes and differential screenings were performed using the forward and reverse subtracted cDNA as probes. After the differential screening, genes with positive results were further confirmed with Northern analysis. Two genes were confirmed by Northern analysis to be differentially expressed in the livers of different lines of chickens. We found that the chicken apoprotein B (apoB) gene was expressed to a greater extent in the livers of L2 line than in the B line chickens, whereas 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (PURH) was expressed to a greater extent in the B line than in the L2 line of chickens (Figure 1). These results suggest that the expression of these genes is altered after the selection for egg production rate in Taiwan Country chickens.

Tissue distribution of chicken genes

The apoB transcript was highly expressed in the liver of laying Taiwan Country chickens (Figure 2). It was not detectable in the muscle, ovary, or oviduct. The PURH transcript was also highly expressed in the liver and to a much lesser extent in the ovary of laying Taiwan Country chickens (Figure 2). It was not detectable in the muscle and oviduct of the Taiwan Country chickens.

DISCUSSION

In mammals, the amphipathic property of apoB makes it a major component of very low density lipoprotein (VLDL) for transporting lipids from the liver to peripheral tissues and formation of low density lipoprotein (Segrest et al., 1992; Boren et al., 1998). Sequence analysis also shows

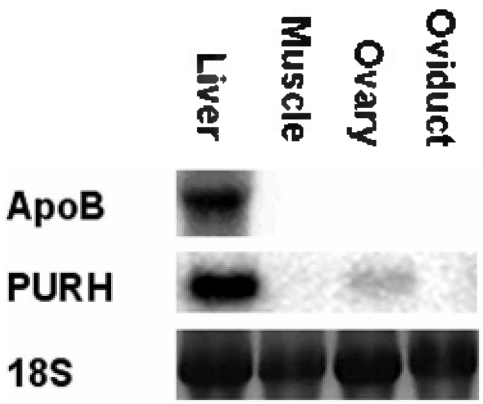

Figure 2. Tissue distribution of apoprotein B (apoB) and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (PURH) transcripts. Total RNA was isolated from liver, muscle, ovary, and oviduct, obtained from 28-week-old L2 and B chickens (n = 8). Twenty μg of total RNA was electrophoresed and transferred to nylon membranes. The membranes were hybridized with chicken cDNA probes for apoB, PURH, and 18S ribosomal RNA (18S). The figure represents the typical expression level of eight birds.

that part of mammalian apoB is homologous to the lipovitellins, one of the major lipid transporting proteins of birds for egg yolk lipid deposition (Baker, 1998; Jolivet et al., 2006). Similar to the mammal, avian apoB is also one of the major components of VLDL for transporting lipids to peripheral tissues (Dixon et al., 1992). Moreover, it is also a major component of yolk VLDL, small size particles for lipid deposition in the yolk, involved in yolk lipid deposition (Walzem et al., 1999). Yolk VLDL contains apoVLDLII that prevents the lipolytic activity by peripheral lipoprotein lipase to ensure the successful deposition of the yolk VLDL components in the yolk of laying birds (Schneider et al., 1990). In the current study, we demonstrated that the apoB mRNA is highly expressed in the liver of laying Taiwan Country chickens similar to what was reported by Dixon et al. (1992) in Leghorn chickens. The results suggest that in the chicken, liver is the major site for production of apoB, although some apoB synthesis can be found in the kidney (Tarugi et al., 1998).

We observed a differential expression pattern of this gene between the two genetic lines (L2 and B), suggesting that the hepatic apoB mRNA concentration can be used as an indication of high egg laying performance (as in L2 chickens). Moreover, because the apoB is involved in transporting lipids for yolk deposition through recognition of a receptor (Nimpf et al., 1989), the higher egg production rate associated with apoB may be a result of better hepatic lipoprotein synthesis in the L2 line than in the B line chickens. Alternatively, the greater expression of the apoB gene in the L2 line chickens may have resulted from co-selection with egg-laying with no functional correlation between the two. No matter which speculation is true, apoB should be useful as a selection marker for the high egg laying Taiwan Country chickens in the future, if the serum apoB protein concentration has the same association as the hepatic apoB mRNA concentration.

The gene PURH is an enzyme with three different enzymatic activities involved in *de novo* purine synthesis in both avian and mammalian species (Aimi et al., 1990; Rayl et al., 1996). The first eukaryote PURH cloned is from an avian hepatic cDNA library (Aimi et al., 1990). Recombinant human PURH protein directly binds to muscle specific splicing enhancer 3 (MSE3) RNA and PURH is the primary determinant that is responsible for the sequence-specific binding activity of this complex (Ryan et al., 2000). These observations strongly suggest that PURH performs a second function as a component of a complex that regulates MSE3-dependent exon inclusion (Ryan et al., 2000). Therefore, the gene is not only simply involved in purine synthesis but also involved in regulating the splicing of MSE3 containing genes. In the current study, we found that the mRNA concentration of PURH in the high egg laying L2 line chickens was much lower than that in the low egg-laying B line chickens. Because the selection for better egg production rate is associated with a lower growth rate, i.e., L2 line chickens grow slower than the B line chickens, the lower expression level of PURH in the L2 line may be associated with its lower growth rate. Alternatively, the greater expression of the PURH gene in the lower egg-laying B line chickens may result from the low egg laying capacity or from the greater growth rate. Regardless, the poor egg laying B line of chickens had greater hepatic PURH mRNA concentration than the higher egg laying L2 line of chickens. The PURH was expressed highly in the liver of laying chickens, as previously reported by Aimi et al. (1990). In mice, the PURH is expressed to a greater extent in the muscle than in any other tissue (Ryan et al., 2000). Therefore, there is a species specific expression pattern of the PURH gene.

Taken together, genes expressed differently in the livers of the two Taiwan Country chicken lines were revealed by the SSH techniques. The association of the expression of these genes with egg-laying performance may be useful as selection markers for Taiwan Country chickens with increased egg-laying performance. The results may extend to selection for egg-laying performance in other chicken breeds. To verify the direct involvement of these genes in egg-laying processes, siRNA technique or gene knockout studies would be techniques of choice. However, in the current study, we have discovered new selection markers for egg-laying performance. Whether these genes are directly involved in egg-laying function requires further experiments.

ACKNOWLEDGEMENT

This work was supported in part by National Science Council. We thank B. H. Liu and H. C. Wang for their technical assistance.

REFERENCES

Abdrakhmanov, I., D. Lodygin, P. Geroth, H. Arakawa, A. Law, J. Plachy, B. Korn and J. M. Buerstedde. 2000. A large database of chicken bursal ESTs as a resource for the analysis of vertebrate gene function. Genome Res. 10:2062-2069.

Aimi, J., H. Qiu, J. Williams, H. Zalkin and J.E. Dixon. 1990. De novo purine nucleotide synthesis: cloning of human and avian cDNAs encoding the trifunctional glycinamide ribonucleotide synthetase-aminoimidazole ribonucleotide synthetase-glycinamide ribonucleotide transformylase by functional complementation in *E. coli*. Nucleic Acids Res. 18:6665-6672.

Baker, M. E. 1988. Is vitellogenin an ancestor of apolipoprotein B-100 of human low-density lipoprotein and human lipoprotein lipase? Biochem. J. 255:1057-1060.

Boren, J., I. Lee, W. Zhu, K. Arnold, S. Taylor and T. L. Innerarity. 1998. Identification of the low density lipoprotein receptor-binding site in apolipoprotein B100 and the modulation of its binding activity by the carboxyl terminus in familial defective apo-B100. J. Clin. Invest. 101:1084-1093.

Carre, W., X. Wang, T. E. Porter, Y. Nys, J. Tang, E. Bernberg, R. Morgan, J. Burnside, S. E. Aggrey, J. Simon and L. A. Cogburn. 2006. Chicken genomics resource: sequencing and annotation of 35,407 ESTs from single and multiple tissue cDNA libraries and CAP3 assembly of a chicken gene index. Physiol. Genomics 25:514-524.

Chang, W. J., C. H. Chen, W. T. K. Cheng and S. T. Ding. 2007. The effect of dietary docosahexaenoic acid enrichment on the expression of porcine hepatic genes. Asian-Aust. J. Anim. Sci. 20:768-774.

Chen, K. L., T. Y. Hsieh and P. W. S. Chiou. 2006. Caponization effects on growth performance and lipid metabolism in Taiwan country chicken cockerels. Asian-Aust. J. Anim. Sci. 19:438-444.

Cheng, C. H., B. R. Ou, T. F. Shen and S. T. Ding. 2006. Effects of dietary algal docosahexaenoic acid oil supplementation on fatty acid deposition and gene expression in laying Tsaiya ducks. Asian-Aust. J. Anim. Sci. 19:1047-1053.

Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate- phenol-chloroform extraction. Anal. Biochem. 162:156-159.

Ding, S. T., R. L. McNeel and H. J. Mersmann. 1999. Expression of porcine adipocyte transcripts: tissue distribution and differentiation *in vitro* and *in vivo*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 123:307-318.

Dixon, J. L., R. Chattapadhway, T. Huima, C. M. Redman and D. Banerjee. 1992. Biosynthesis of lipoprotein: location of nascent apoAI and apoB in the rough endoplasmic reticulum of chicken hepatocytes. J. Cell Biol. 117:1161-1169.

Hsu, J. M., P. H. Wang, B. H. Liu and S. T. Ding. 2004. The effect of dietary docosahexaenoic acid on the expression of porcine lipid metabolism-related genes. J. Anim. Sci. 82:683-689.

Jolivet, P., C. Boulard, V. Beaumal, T. Chardot and M. Anton. 2006. Protein components of low-density lipoproteins purified from hen egg yolk. J. Agric. Food Chem. 54:4424-4429.

Lee, Y. P. 2006. Taiwan Country Chicken: a slow growth breed for eating quality. Proceedings Symposium 2006 Scientific Cooperation in Agriculture between Council of Agriculture (Taiwan, ROC) and Institut National de la Recherche Agronomique (France). pp. 121-132. Tainan, Taiwan.

Lee, Y. P., L. T. Yeh and H. H. Huang. 1997. A study on the production system of Taiwan Country chicken: growth traits of three-strain cross Country chickens. J. Chin. Anim. Sci. 26:271-284.

Nimpf, J., M. Radosavljevic and W. J. Schneider. 1989. Specific post-endocytic proteolysis of apolipoprotein-B in oocytes does not abolish receptor recognition. Proc. Natl. Acad. Sci. USA 86:906-910.

Rayl, E. A., B. A. Moroson and G. P. Beardsley. 1996. The human purH gene product, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase. Cloning, sequencing, expression, purification, kinetic analysis, and domain mapping. J. Biol. Chem. 271:2225-2233.

Ryan, K. J., N. Charlet-B and T. A. Cooper. 2000. Binding of PurH to a muscle-specific splicing enhancer functionally correlates with exon inclusion *in vivo*. J. Biol. Chem. 275:20618-20626.

SAS. 2001. SAS User's Guide: Statistics. SAS Institute, Inc., Raleigh. NC.

Segrest, J. P., M. K. Jones, H. De Loof, C. G. Brouillette, Y. V. Venkatachalapathi and G. M. Anantharamaiah. 1992. The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. J. Lipid. Res. 33:141-166.

Schneider, W. J., R. Carroll, D. L. Sevenson and J. Nimpf. 1990. Apolipoprotein VLDL-II inhibits lipolysis of triglyceride-rich lipoproteins in the laying hen. J. Lipid. Res. 31:507-503.

Spike, C. A., N. Burnstead, L. B. Crittenden and S. J. Lamont. 1996. RFLP mapping of expressed sequence tags in the chicken. J. Hered. 87:6-9.

Sun, D. X., W. Dong, Y. Ying and Z. Yuan. 2005. Cloning and characterization of liver cDNAs that are differentially expressed between chicken hybrids and their Parents. Asian-Aust. J. Anim. Sci. 18:1684-1690.

Tarugi, P., G. Ballarini, B. Pinotti, A. Franchini, E. Ottaviani and S. Calandra. 1998. Secretion of apoB- and apoA-I-containing lipoproteins by chick kidney. J. Lipid. Res. 39:731-743.

Tirunagaru, V. G., L. Sofer, J. Cui and J. Burnside. 2000. An expressed sequence tag database of T-cell-enriched activated chicken splenocytes: sequence analysis of 5251 clones. Genomics 66:144-151.

Walzem, R. L., R. J. Hansen, D. L. Williams and R. L. Hamilton. 1999. Estrogen induction of VLDLy assembly in egg-laying hens. J. Nutr. 129:467S-472S.

Wang, P. H., B. H. Liu, Y. H. Ko, Y. C. Li and S. T. Ding. 2004. The expression of porcine adiponectin and stearoyl coenzyme A desaturase genes in differentiating adipocytes. Asian-Aust. J. Anim. Sci. 17:588-593.

Wang, H. C., Y. H. Ko, H. J. Mersmann, C. L. Chen and S. T. Ding. 2006. The expression of genes related to adipocytes in pigs. J. Anim. Sci. 84:1059-1066.

Yen, C. F., Y. N. Jiang, T. F. Shen, I. M. Wong, C. C. Chen, K. C.

Chen, W. C. Chang, Y. K. Tsao and S. T. Ding. 2005. Cloning and expression of the genes associated with lipid metabolism in Tsaiya ducks. Poult. Sci. 84:67-74.

Yen, C. F., H. W. Lin, J. C. Hsu, N. C. Lin, T. F. Shen and S. T. Ding. 2006. The expression of pituitary gland genes in laying geese. Poult. Sci. 85:2265-2269.

Yen, C. J. 2004. Comparison of follicle growth rate and oviposition interval among commercial Taiwan Country chicken, selected Taiwan Country chicken and Single-Comb White Leghorn. Master Thesis. National Chung-Hsing University, Taichung, Taiwan.